RESISTANCE TO THE POTATO CYST NEMATODE (*GLOBODERA ROSTOCHIENSIS*) OF TETRAPLOID POTATO CLONES WITH DIFFERENT GENETIC BACKGROUNDS

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Summary. Fifteen new potato clones were screened to pathotype Ro2 of the potato cyst nematode, *Globodera rostochiensis*. One dm^3 clay pots were filled with soil infested with 17 eggs of the nematode/cm^3 and six of them were planted with tubers of each clone and arranged in a glasshouse at 18-22 °C. When thirty-five days old, all plants were uprooted and nematode infestation of the roots evaluated according to a visual score and by extracting all nematode developmental stages. All clones were invaded by a large number of nematode specimens. However, in seven potato clones (S90-1575-5, S90-157-19, S90-157-27, S90-157-28, S90-157-49, S90-164-1, S90-164-7) most of the nematodes (71.4-94.8%) remained at the second stage and none or only a few developed further to become third, fourth and adult female stages and cysts. These clones were considered resistant.

Potato is a very important crop in several Italian regions. Due to favourable environmental conditions, it can often be cultivated throughout the year. Three different planting seasons can be identified: winter (tubers are planted in November-December), spring (tubers are planted in January-March), and autumn (tubers are planted in August-September). In some southern areas potatoes can be grown twice a year, in spring and autumn.

As well as the availability of cultivars adapted to the environmental conditions of the Mediterranean area, one of the main problems related to potato cultivation in Italy is the presence of several pathogens and pests (Frusciante and Ranalli, 1999). Among them, the cyst nematodes *Globodera rostochiensis* (Woll.) Behrens and *Globodera pallida* (Stone) Behrens, are widespread and cause damage to potato all over the country (Greco *et al.*, 1993), especially in areas where crop rotation is only short term or not at all used. The latter occurs more often in the areas where early potatoes are cultivated. However, in the August-September cycle, the soil temperature (> 25 °C) suppresses egg hatching at an early potato stage, but as soon as the soil temperature drops to about 20 °C, in late September-early October, the nematodes becomes active and severe damage can be observed by late October-early November. The nematode causes yield loss whenever its population densities exceed 1.9 eggs/g soil (Greco *et al.*, 1982; Seinhorst, 1982).

Although nematicides are effective against cyst nematodes, the use of resistant cultivars is preferred because it is environmentally acceptable and economic. Unfortunately, several pathotypes are known of both *G. rostochiensis* and *G. pallida* (Canto and Schurrah, 1977; Kort *et al.*, 1977; Fleming and Powers, 1998). Moreover, only a few potato cultivars are resistant to all pathotypes of the same cyst nematode, most being only resistant to pathotype Ro1 (Whitehead and Turner, 1998). In this article, a number of new potato clones with different genetic backgrounds and suitable for potato cultivation in southern Italy were evaluated to ascertain their reaction to pathotype Ro2 of *G. rostochiensis*.

MATERIALS AND METHODS

Plant material. Plant material, *Solanum tuberosum* L., was developed within the potato breeding programme carried out at the University of Naples. It included 15 tetraploid clones coming from the following five cross combinations: NY 76 x Atzimba (family code S90-157), Ukama x Carmine (family code S90-164), NY 76 x V 2 (family code S90-165), DTO 28 x V 2 (family code S90-151), Chiquita x Conchita (family code 163), CEW69.1 x LT7 (family code 154), and NY 76 x 7XY.1 (family code S90-159). Clone NY 76 was developed by the potato breeding program of Cornell University (USA), and is resistant to *G. rostochiensis*. Varieties V 2, 7XY.1, Chiquita, Conchita, CEW69.1, LT7 and DTO 28 come from the collection of the International Potato Center (Lima, Peru) and were selected in Italy as TPS parents. Carmine and Ukama are commercial European cultivars. They are all susceptible to *G. rostochiensis* with the sole exception of Ukama which is resistant. To produce tubers, plants were grown in Camigliatello Silano (CS) during the summer of 1998, using the standard potato field procedures for the area.

Screening for resistance. The population of *Globodera rostochiensis* (Woll.) Behrens, previously identified as pathotype Ro2 (Kort *et al.*, 1978; Greco *et al.*, 1999), was collected from an infested field at Molfetta (Province of Bari) soon after harvest of potato cv. Spunta. This pathotype was selected because it was found to be the most common in Italy. Cysts were extracted from dried soil using a Fenwick can, dried and thoroughly mixed with about 2 kg of river sand. To estimate the nematode population density in the medium, five samples of 10 g each were poured separately on to a 25-mesh sieve nested on a 60-mesh one and sprayed with tap water. Cysts and soil debris retained on the 60-mesh sieve were collected and the cysts separated under a stereomicroscope and crushed according to Bijloo's modified method (Seinhorst and Ouden, 1966) to count their egg content and estimate the nematode population density. A suitable amount of this soil was then thoroughly mixed with the required potting soil (89% sand) to obtain a nematode density of 17 eggs/cm^3. The infested soil was then used to fill 108 clay pots, each containing 1 dm^3 soil.

Three tubers for each of the fifteen potato clones were supplied by the University of Naples and each cut in half to obtain six sprouting potato tuber pieces which were planted in six separate pots. Potato cv. Spunta was used as control. There were six replicates per potato clone, arranged in a randomised block design on a bench in a glasshouse maintained at 18-22 °C. The pots were irrigated and fertilised as required. Twelve additional pots were planted with potato cv. Spunta and used to monitor the development of the nematode on the roots. When several golden nematode females had developed on the roots (35 days after plant emergence), the test was terminated. Then all plants were uprooted, the roots gently washed in running water and their infestation estimated visually according to a 0-5 rating scale based on numbers of nematode females and cysts (Di Vito *et al.*, 1988). Thereafter, excess water was eliminated and the roots were weighed, cut into pieces 0.5 cm long and all nematode stages extracted according to Coolen's method (Coolen, 1979). Nematodes were then counted and classified according to their developmental stage. The per cent of different life stages, on the total numbers of specimens, were also calculated.

All data were statistically analysed and means compared with Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Nematode infestation of the roots of the test cultivar Spunta was good and the results can therefore be considered reliable. Moreover, there is general agreement in the reaction of the various potato clones to *G. rostochiensis* as estimated according to the visual infestation score or the number of specimens of different nematode stages extracted from the roots. The only uncertainty is with clone S90 164-1, of which three tubers germinated later; the plants had fewer roots at the end of the test, and the roots of one plant were heavily infested with the nematode.

There was little difference in the number of total nematode specimens extracted from the roots of different clones (Table I). However, the roots of two clones (S90-159-6 and S90-164-8) had significantly more nematode specimens than the roots of the control cultivar Spunta. A few differences were also observed in the percentage of nematode males (third and fourth stage juveniles and adults), but they were of little value for estimating the reaction of the clones to the nematode. In contrast, percentages of other developmental stages of the nematode revealed significant differences among the tested clones. A large proportion of nematodes remained at second stage juveniles in clones S90-164-7 (94.8%), S90-157-49 (92.8%), S90-157-19 (90.2%) and also in clones S90-157-5 (78.2%), S90-157-28 (76.3%) and S90-164-1 (74%). This clearly indicates that these clones possess resistance to pathotype Ro2 of *G. rostochiensis*. Further confirmation of this finding is given by the per cent of nematode specimens which continued in their development. No third or fourth stage females were found in the roots of clones S90-157-19 and S90-157-49 and only a few in clone S90-164-7. Per cent of these juvenile stages were also small (3.0-5.9) in the roots of clones S90-157-5, S90-157-27, S90-157-28 and S90-164-1. Percentages of these female stages in the remaining clones were in the range 11.95-32.2 and differed to some extent among one other. The same trend was observed in adult females and cysts of the nematode. These stages were absent in clones S90-157-19, S90-157-49 and S90-164-7, with to a small proportion (7.1-10.9%) in clones S90-157-5, S90-157-27, S90-157-28, and a substantial proportion (26-67.9%) in the remaining clones (Table I).

According to these results seven clones (S90-157-19, S90-157-49, S90-157-5, S90-157-27, S90-157-28, S90-164-1, S90-164-7) did not allow or greatly suppressed reproduction of the nematode and can therefore be considered resistant or very resistant to pathotype Ro2 of *G. rostochiensis*. The nematode reproduced well in all the remaining clones which thus are considered susceptible, although differences were observed among them. The large number of nematode specimens observed in the resistant clones clearly indicates that the resistance mechanism is only activated after the nematode has penetrated the roots and not earlier on egg hatching (Evans, 1983; Forrest and Phillips, 1984). Moreover, resistance does not seem to affect the further development of second stage juveniles into different male stages. Usually, screening of a large amount of breeding material is done by visual scoring based on numbers of females and cysts of the nematode (Fleming, 1998). Extending this standard practice, we also extracted and counted all nematode life stages to obtain further insights into the reaction of the various potato clones to the nematode. Therefore, these results are very reliable and appear very promising in that the seven resistant potato clones also possess agronomic traits which make them useful for cultivation in southern Italy and, in general, in the Mediterranean area. In fact, most of them are early and this aspect, associated with the possibility of growing potatoes in a winter-spring cycle, would make their production very competitive (Frusciante and Ranalli, 1999).

Table I. Specimens of *Globodera rostochiensis* extracted and infestation rates of roots of new potato clones.

Clone	Nematodes in 10 g roots	% nematode developmental stages				Infestation rate[1]	Reaction type
		Second stage Juveniles	Males	Third and fourth Stage females	Females and cysts		
S90 157-5	993 abc	78.2 b	11.1 abc	3.1 ab	7.5 ab	0.5 a	Resistant
S90 157-19	1646 abcd	90.2 b	9.8 abc	0.0 a	0.0 a	0.0 a	Resistant
S90 157-27	925 abc	71.4 b	14.2 abcd	5.9 abcd	8.4 ab	0.8 ab	Resistant
S90 157-28	1105 abc	76.3 b	11.1 abc	5.5 abcd	7.1 ab	0.5 a	Resistant
S90 157-49	1897 bcd	92.8 b	7.1 ab	0.0 a	0.0 a	0.0 a	Resistant
S90 157-76	1217 abcd	3.5 a	11.7 abc	16.8 def	67.9 f	4.7 e	Susceptible
S90 164-1	1336 abcd	74.0 b	10.8 abc	4.3 abc	10.9 abc	0.7 ab	Resistant
S90 164-7	1026 abc	94.8 b	5.1 a	0.1 a	0.0 a	0.0 a	Resistant
S90 164-8	2098 cd	7.5 a	27.1 def	16.0 cdef	49.3 def	4.3 de	Susceptible
S90 151-1	694 a	21.2 a	17.4 bcde	13.2 bcdef	48.3 def	3.8 cde	Susceptible
S90 151-14	1924 bcd	6.7 a	35.3 f	18.5 ef	39.5 de	4.3 de	Susceptible
S90 154-29	1778 abcd	19.6 a	18.9 bcde	11.9 bcde	49.5 def	3.7 cde	Susceptible
S90 159-6	2492 d	20.9 a	20.7 cdef	32.2 g	26.0 bcd	2.3 bc	Susceptible
S90 163-6	1143 abc	9.4 a	31.7 ef	23.7 efg	32.8 cde	2.8 cd	Susceptible
S90 165-4	774 ab	21.8 a	18.5 bcde	13.9 bcdef	45.7 def	3.1 cde	Susceptible
Cv. Spunta	858 ab	4.2 a	15.7 abcd	24.5 fg	55.4 ef	4.2 de	Susceptible

Figures flanked by the same letter are not significantly different according to Duncan's Multiple Range Test.
[1] Visual rating based on numbers of nematode females and cysts on the roots: 0 = 0, 1 = 1-2, 2 = 3-5, 3 = 6-20, 4 = 21-50,and 5 = > 50 females and or cysts per root. Plants whose roots were rated 0-2 were considered resistant.

Interestingly, out of the seven clones resistant to *G. rostochiensis*, five originated from the cross combination NY 76 x Atzimba and two from Ukama x Carmine. These results are not surprising, considering that the female parents NY 76 and Ukama are resistant to this cyst nematode. NY 76 comes from *S. andigena*, one of the best sources of resistance to nematodes within potato species (Ellenby, 1952; Phillips, 1994; Dale and de Scurrah, 1998). It can be hypothesised that the resistant clones have introgressed a) the dominant major gene conferring the resistance from the resistant parent and b) minor genes helping to complete the resistance from either one or both parents (Ross, 1986).

Research is in progress to ascertain their reaction to other pathotypes of *G. rostochiensis* and *G. pallida*, with the final aim of assessing their impact on nematode population dynamics and hence their suitability to Italian cropping conditions.

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